Inadequate food intake at high temperatures is related to depressed mitochondrial respiratory capacity

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ABSTRACT

Animals, especially ectotherms, are highly sensitive to the temperature of their surrounding environment. Extremely high temperature, for example, induces a decline of average performance of conspecifics within a population, but individual heterogeneity in the ability to cope with elevating temperatures has rarely been studied. In this study, we examined inter-individual variation in feeding ability and consequent growth rate of juvenile brown trout *Salmo trutta* acclimated to a high temperature (19˚C), and investigated the relationship between these metrics of whole-animal performances and among-individual variation in mitochondrial respiration capacity. Food was provided *ad libitum* yet intake varied ten-fold amongst individuals, resulting in some fish losing weight whilst others continued to grow. Almost half of the variation in food intake was related to variability in mitochondrial capacity: low intake (and hence growth failure) was associated with high leak respiration rates within liver and muscle mitochondria, and a lower coupling of muscle mitochondria. These observations, combined with the inability of fish with low food consumption to increase their intake despite *ad libitum* food levels, suggest a possible insufficient capacity of the mitochondria for maintaining ATP homeostasis. Individual variation in thermal performance is likely to confer variation in the upper limit of an organism's thermal niche and in turn affect the structure of wild populations in warming environments.

Key words: ecophysiology, food intake, global warming, proton leak, respiratory control ratio, respiration rate.

INTRODUCTION

Increasing temperature due to climate change is expected to have profound consequences across multiple levels of biological organisation, from the three-dimensional structure of proteins to the abundance and distribution of species (Sunday et al., 2011). While the extreme outcome of high temperatures is the rapid death of the organism, occurring within minutes to hours (Somero, 2010), there are many sub-lethal costs of high temperatures on animal function, including decreased locomotory capacity (Steinhausen et al., 2008), food intake (Van Doorslaer and Stoks, 2005), growth (Bermudes et al., 2010) and reproduction (Donelson et al., 2014). Given the gradual rise in global temperature (Pachauri et al., 2014), it is likely that declines in performance that occur at sub-lethal rather than upper-lethal temperatures will be a more realistic threshold for the long-term persistence of individual species (Farrell et al., 1996; Somero, 2010).

Investigations into the biological effects of global warming have generally focused on the average performance of species (Seebacher et al., 2015; Skelly et al., 2003), populations (Eliason et al., 2011) or experimental groups (Kingsolver et al., 2004). While these approaches are clearly useful, recent research has recognized the importance of accounting for individual heterogeneity in predicting responses to global warming since some individuals perform much better than others within the same thermal environment (Calosi et al., 2013; Careau et al., 2014; Kingsolver et al., 2004). Thus, individual variation in the ability to cope with warming conditions is likely to directly influence the potential for species to evolve adaptations for a warming world, so affecting their abundance and distribution (Vindenes and Langangen, 2015).

Here we examine individual variability in the ability to cope with high but non-lethal temperatures, adopting an integrative approach that relates whole-organism performance to mitochondrial respiratory capacities. In biology, one of the most fundamental processes is the uptake and transformation of resources into a form of energy that can be utilised by cells. The mitochondria occupy a pivotal position between resource uptake and whole-organism performance through their primary role in ATP production. To maintain cellular function, the rate of ATP production must, at least, match the rate at which ATP is used by processes including locomotion, growth, reproduction, in addition to those maintaining cellular homeostasis (Willmer et al., 2005). Given that variation in mitochondrial respiratory capacities is known to influence animal performance (Bottje and Carstens, 2009; Coen et al.,

2012; Rawson et al., 1996; Salin et al., 2015) and that key mitochondrial processes are exquisitely sensitive to temperature (Blier et al., 2013), it can be reasoned that mitochondrial function may influence individual variation in performance at high temperatures (Schulte, 2015).

In ectotherms, one consequence of higher temperatures is an increase in oxygen demand that is independent of the rate of mitochondrial ATP production (Sommer and Pörtner, 2004). A proportion of the oxygen consumed by the mitochondria is not used to produce ATP but rather to counteract the leak of protons across the mitochondrial inner membrane (Rolfe and Brand, 1997). The rate of proton leak increases disproportionately with increasing temperature, so that at higher temperatures a decreasing proportion of the consumed oxygen is used to produce ATP (Abele et al., 2002; Brooks et al., 1971; Chung and Schulte, 2015; Fangue et al., 2009; Guderley and Johnston, 1996). An increase in oxygen consumption can compensate for the rise in proton leak and help maintain ATP production (Rolfe and Brand, 1997). However, oxygen consumption cannot increase indefinitely, and above a threshold temperature the proportion of oxygen dedicated to the production of ATP will drop, resulting in mitochondria being unable to maintain ATP homeostasis. In addition, the ability to compensate for an increasing proton leak may decline in warmer condition since oxygen solubility in water declines with increasing temperature (Pörtner, 2001; but see Verberk et al., 2011). It is conceivable that high temperatures may place greater constraints on those individuals with relatively high mitochondrial proton leak respiration. Therefore, variation in mitochondrial proton leak may influence individual differences in performance at elevating temperatures, but this remains unexplored.

Another major cellular consequence of higher temperatures is the loss of function of mitochondria (Guderley and St-Pierre, 2002; O'Brien et al., 1991; Pörtner et al., 1999; Somero, 2010; St-Pierre et al., 2000). Changing mitochondrial function can be tracked experimentally through measurement of the respiratory control ratio (RCR), an index of mitochondrial coupling (Brand and Nicholls, 2011; Kayes et al., 2009) – calculated as the ratio of respiration supporting ATP synthesis (OXPHOS) to the respiration required to offset proton leak (LEAK) (Estabrook, 1967). A low RCR indicates that the mitochondria have a low capacity for phosphorylating oxidation relative to the oxidation required to offset the proton leak (Brand and Nicholls, 2011). The RCR may be depressed with temperature increases because of a loss in function of enzymatic complexes (Somero, 2010) and/or an excessive LEAK without a parallel increase in OXPHOS capacity. Therefore, RCR

encapsulates the main functions of mitochondria susceptible to temperature (Fangue et al., 2009; Iftikar et al., 2014; Iftikar et al., 2015; Pörtner et al., 1999), and can be used to reveal thermally-induced changes in the mitochondria; a drop in RCR at high but sub-lethal temperatures being a determinant of the failure of tissue function (Iftikar et al., 2014).

The purpose of the present study was to test two non-exclusive hypotheses that at high but non-lethal temperatures, whole-organism performance will be poorer in individuals that have: i) higher mitochondrial LEAK respiration rates and ii) a lower RCR. To test these hypotheses, we assessed the relationship between the mitochondrial respiratory capacities of liver and white muscle (tissues likely to influence whole-organism performance (Bottje and Carstens, 2009; Eya et al., 2012; Rawson and Friedman, 1994)), and feeding ability and growth rate in juvenile brown trout *Salmo trutta* maintained near their upper lethal thermal limit, where average food intake starts to decline (Elliott, 1976).

MATERIAL AND METHODS

Animal care and thermal acclimation

Brown trout *Salmo trutta*, the offspring of wild parents collected from the River Tweed, Scotland, were hatched in aquaria at the University of Glasgow in spring 2014 and reared in communal stock tanks. From summer 2014, all fish were maintained under a 8L: 16D photoperiod at 10°C in individual compartments within a stream tank system that allowed individual daily feeding with trout pellets (EWOS, West Lothian, UK) while maintaining fish in flowing water. The first phase of thermal acclimation consisted in maintaining the fish under these conditions (10°C) for 7 weeks. They were then gradually acclimated to the high testing temperature (19°C) by means of a sequence of three step increases of 3°C over a 48h period, each separated by a 5-week period of stability to ensure physiological adjustment to the change in temperature (Bouchard and Guderley, 2003). Throughout this 22-week acclimation period fish were fed twice per day with trout pellets on a specific ration. The ration was equivalent to 80% of the individual fish's maximum food intake calculated according to (Elliott, 1976), based on acclimation temperature and individual body mass. All fish consumed their entire daily ration at the two lower acclimation temperatures (i.e. 10 ˚C and 13 ˚C), so the variation in feeding capacities at the high testing temperature (see below) was unrelated to any difference in feeding capacities of the fish at the start of the acclimation period.

Food intake and growth

Food intake and growth were recorded simultaneously over a 2-3 week measurement period (see below) starting after the 5-week acclimation at 19°C. This temperature was chosen because it is approaching the upper temperature at which their average food intake and growth decline considerably but is safely below the temperature which causes acute death (above 22˚C) (Elliott, 1976). At the start of the experiment, all individuals were briefly anaesthetised (50 mg/l benzocaine diluted in water), and measured for fork length (± 1 mm) and body mass (± 1 mg). All fish were fasted for two days beforehand to ensure their guts were empty. Fish were then randomly separated into 3 batches of 10-13 fish for the food intake and growth measurements, with the starting time of the three batches staggered by a day, since only 3-5 fish per day could be processed for mitochondrial respiratory capacities (measured at the end

of the growth trial); the durations of the feeding and growth trials within each batch were 11, 14 or 17 days and 14, 17 or 20 days, respectively. There is a 3-day difference in duration between the feeding and growth trials because the recording of food intake started a day after the initial body size measurement (to allow time to recover from the anaesthetic) and ended 2 days before the final body size measurement.

During the time period that food intake was recorded, fish were fed *ad libitum* with a known number of pellets twice a day at 0900 and 1600 h. Prior to each meal, pellets remaining uneaten from the previous meal were counted and removed by siphon along with faecal matter in order to maintain water quality. Daily food intake was calculated as the difference between the number of pellets offered and the number remaining per day, and was expressed in terms of its mass (one pellet weighing 1.44 mg). At the end of the feeding trial, fish were again fasted for two days, culled and their fork length and body mass re-measured before liver and white muscle were collected to determine mitochondrial respiratory capacity (see below). Specific growth rate in either length or mass was defined as:

$$\text{specific growth} = \frac{\ln \text{final fish size} - \ln \text{initial fish size}}{\text{days elapsed}} * 100$$

Mitochondrial respiratory capacities

Immediately after sacrifice of the fish, the liver and a subsample of white muscle were weighed and preserved in ice-cold MiR05 respirometry buffer (0.5mM EGTA, 3.0mM $MgCl_2.6H_2O$, 20mM Taurine, 10mM KH_2PO_4, 20mM HEPES, 110 mM D-sucrose, 60 mM lactobionic acid, 1g L^{-1} bovine serum albumin essentially free fatty acid, pH 7.3) for analyses of mitochondrial respiratory capacities. The technique for preparation of the white muscle and liver shredding was adapted from (Kuznetsov et al., 2002; Kuznetsov et al., 2008; Larsen et al., 2014). Briefly, tissues were cut using micro-dissecting scissors to obtain a homogenous solution with particle size lower than 0.5 mm (tested by pipetting through 1mL tip) in 1mL MiR05. The shredded homogenates were then diluted further in MiR05 to obtain the desired final concentration (mean ± SE: 8.5 ± 0.3 mg mL^{-1} for the liver and 20.0 ± 0.1 mg mL^{-1} for the white muscle). The entire procedure was carried out at 4°C, and completed within 15 min of the fish being culled. The quality and permeability of our tissue preparations by shredding were compared with chemical (with addition of saponin) and mechanical (by homogenising

with a potter Elvehjem) permeabilization of the muscle and liver, respectively (as done in (Kuznetsov et al., 2002) for liver, (Larsen et al., 2014) for muscle). These pilot studies demonstrated that plasma membrane permeability was similar irrespective of the preparation method, and the oxidative capacities and integrity of the mitochondrial membrane for shredder homogenate similar to the mechanical homogenisation and better than the chemical permeabilization (data not shown). The tissue preparation by shreddering used here allowed a rapid and efficient liver and muscle preparation, with no loss of tissue, while maintaining the quality of the mitochondria.

Mitochondrial respiration in samples of liver or muscle homogenate was measured at 19°C using two Oxygraph-2k high resolution respirometers (Oroboros Instruments, Innsbruck Austria) running in parallel, each with two independent chambers, although one electrode used for the respirometer devoted to liver samples failed during the experiment (all the data collected from this electrode have been excluded from analyses). The oxygen electrodes were calibrated at two points: air-saturated MiR05 and zero oxygen after sodium dithionite addition. Oxygen concentration (nmol mL^{-1}) was recorded using DatLab software (Oroboros Instruments).

A sequential substrate/inhibitor addition protocol, adapted from (Pesta and Gnaiger, 2012), was run simultaneously for each tissue in duplicate and was completed within 2 hours. The protocol was as follows: first the tricarboxylic acid cycle was reconstituted by adding pyruvate (5 mM) and malate (0.5 mM) to support electron entry to complex I, and succinate (10 mM) to support electron entry to complex II. Oxidative phosphorylation capacity – OXPHOS respiration - was reached by adding a saturating concentration of ADP (1mM ADP with 6 µM Mg^{2+}) and then Cytochrome c (Cytc - 10 µM). Cytc was added as a quality control, with a Cytc stimulation of OXPHOS respiration rate below 15% indicating acceptable outer mitochondrial membrane integrity (Kuznetsov et al., 2002). Addition of Cytc to the white muscle preparation led to a statistically significant but biologically small elevation in the respiration rate (mean increase of 3.4 %, paired $t = -14.805$, $n = 35$, $p < 0.001$). Adding Cytc to the liver preparation led to an even smaller increase in the respiration rate (2.9 %, paired $t = -12.295$ $n = 35$, $p < 0.001$), again indicating that the tissue preparation was good. For both tissues, respiration rates in the presence of ADP plus Cytc were used when describing phosphorylating respiration and in the calculation of the respiratory control ratio. LEAK respiration, i.e. the oxidation to compensate for the proton leak across the mitochondrial membrane in the absence of phosphorylation, was induced by adding

oligomycin (2 µg mL), an inhibitor of ATP synthase. Addition of complex I inhibitor (0.5 µM rotenone) and complex III inhibitor (2.5 µM antimycin A) determined residual (i.e. biological but non-mitochondrial) oxygen consumption, which was then subtracted from all other values. Finally, Cytochrome c oxidase (COX) respiration, a measure of the mitochondrial density of the tissues (Larsen et al., 2012), was measured by adding ascorbate (8 mM) and N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride (0.5 mM).

To avoid any limitation of oxygen diffusion into cells (Gnaiger et al., 1998) the sequential protocol was run under hyperoxygenation (350-550 µM for the muscle and 100 – 550 µM for the liver, ranges within which the tissues were found not to be oxygen sensitive). Pure oxygen was added to the respirometry chamber at the beginning of the titration and again before the addition of oligomycin. Mass-specific oxygen consumption at each step in the protocol was averaged over several minutes after stabilisation of the flux, and expressed as O_2 per sec^{-1} per mg^{-1} wet weight of tissue and corrected for instrumental background oxygen flux arising from oxygen consumption of the oxygen sensor and back-diffusion into the chamber (Pesta and Gnaiger, 2012). The respiratory control ratio (RCR) was calculated as the ratio of phosphorylating respiration in presence of cytochrome c (OXPHOS) to leak respiration (LEAK).

Statistical analysis

We first tested whether the mitochondrial respiratory capacities (LEAK, OXPHOS and COX activity) of the liver and/or muscle together with the fish body length explained individual variation in food intake. Since mitochondrial density of the tissue can influence both mass-specific LEAK and OXPHOS rates, we first corrected for mitochondrial density by calculating residuals from separate regressions relating LEAK and OXPHOS to COX activity for each tissue (SI method and SI table S2); residuals (hereafter $LEAK_{cox}$ and $OXPHOS_{cox}$) were used instead of the fraction of mass-specific mitochondrial respiration rates per COX activity since mass-specific mitochondrial respiration rates were not isometrically related to COX activity (SI table S2). Preliminary analyses show that mitochondrial capacities were not affected by the day of measurement, so day was not included as a covariate in the final model.

We also tested whether the degree of mitochondrial coupling, as determined by RCR, together with fish length explained individual variation in food intake; this had to be examined in a

separate linear model to prevent problems associated with multicollinearity since RCR was correlated with LEAK (e.g. RCR versus leak respiration in liver: Pearson r = -0.468; in muscle: Pearson r = -0.527). We then used this same approach to test whether the mitochondrial capacities (LEAK, OXPHOS and COX activity) of the liver and/or muscle together with food intake explained individual variation in specific growth rate in mass and size. As above, LEAK and OXPHOS were considered in separate models from those considering RCR. Preliminary analysis showed that specific growth in body size was not affected by the initial size, so initial size was not included as a covariate in the final model. The pattern of the results of the analyses of growth rate were the same whether growth was measured in terms of mass or length, so only those for growth in mass are reported in the text (see SI table S2 and figure S1 for analysis of growth in length).

The duration of the feeding and growth trials were included as potential covariates in each model, but were not significant so were subsequently removed. Variance inflation factors for all predictors in each regression analysis were below 3. The normality of the regression residuals was validated for all models using the Shapiro-Wild statistic. Partial eta-squared (η_p^2) values were calculated as a measure of effect size for each significant predictor variable in each analysis. All analyses are based on a sample size of 35 fish. All statistical analyses were performed in IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA); the level of significance set to $p < 0.05$.

RESULTS

Daily food intake at 19°C was highly variable among individuals, ranging from 1.1 ‰ to 13.4 ‰ of body mass. Food intake (mg d^{-1}) was positively related to fish body length (η_p^2 = 0.435, Table 1), as predicted. However, mitochondrial respiratory capacities varied up to 2-fold across individuals for each tissue (Table 2), and 48% of the variation in food intake after controlling for body size was explained by individual differences in respiratory capacities of both the liver and the white muscle mitochondria. Specifically, food intake was lower in fish with higher $LEAK_{cox}$ respiration in both liver and muscle mitochondria (η_p^2 = 0.204 and η_p^2 = 0.135, Figure 1a and b, respectively, Table 1) and lower in fish with lower $OXPHOS_{cox}$ of muscle mitochondria (η_p^2 = 0.143, figure 1c, Table 1). As a result, daily food intake was positively linked to RCR in muscle (η_p^2 = 0.119, Figure 1d, Table 1) but not in liver (Table 1). Food intake was not related to $OXPHOS_{cox}$ in liver mitochondria, or to mitochondrial density (COX activity) in either the liver or the muscle (Table 1).

The specific growth rate of the trout at 19°C differed significantly among individuals, ranging from -1.04 % loss to +2.48 % gain in mass per day (Figure 2). Specific growth in mass was a positive function of food intake (Figure 2) and so indirectly related to mitochondrial properties, although these had no additional effect on growth once food intake was taken into account (Table 3).

DISCUSSION

Individual variation in performance at high temperatures has been recognized recently as an important predictor of population dynamics in the context of global warming (Calosi et al., 2013; Vindenes and Langangen, 2015). However, relatively little is known about the degree to which whole-animal and sub-cellular metrics of performance can co-vary among individuals under the same environmental conditions. Our results demonstrate that among brown trout raised under the same conditions at a high but sub-lethal temperature (19˚C), there was substantial variation in food intake and growth: some individuals were able to maintain food intake to support growth whereas others ate little and lost weight. The differences among individuals in feeding capacity – and indirectly in growth - were largely explained by the mitochondrial capacities of both their liver and white muscle. Specifically, individuals that ate the least, and thus exhibited the slowest growth, displayed a higher leak respiration and a lower mitochondrial coupling (as indicated by a lower RCR) than individuals that ate more under the same conditions.

This study provides the first example in non-domesticated animals of how variability in food intake and growth among conspecifics under the same environmental conditions is associated with the functioning of their mitochondria. While it has previously been shown that different species or treatment groups acclimated to different temperatures can exhibit variability in their LEAK respiration and RCR in mitochondria at high temperatures (Chung and Schulte, 2015; Fangue et al., 2009; Iftikar et al., 2014; Khan et al., 2014), we show that the individual heterogeneity in mitochondrial capacities was independent of environmental conditions. All the animals in this study were maintained from the egg stage onwards under the same standardized conditions, and were given an equal ration (proportional to body size) for 5 months before the experiment. Thus, we can rule out the possibility that water quality, food and oxygen availability or any other environmental factor played a role in the observed variation.

The relationships between feeding capacities and mitochondrial properties we observed, while correlative, contrast with the finding that an experimental restriction of food intake can cause a decrease in LEAK (Bevilacqua et al., 2004) and increase RCR (Lanza et al., 2012). The latter studies were in endotherms, which could potentially explain some of the difference. However, our results are in line with the growing evidence that mitochondrial respiratory capacities are determinants of animal success or failure at high temperatures (Blier et al.,

2013; Fangue et al., 2009; Iftikar and Hickey, 2013; Khan et al., 2014). Ectothermic aquatic organisms with a higher oxygen requirement are expected to suffer more from the drop in dissolved oxygen concentrations at high environmental temperatures, compared with individuals that require less oxygen (Pörtner, 2002). The mismatch between the demand for oxygen and the oxygen supply to the mitochondria is therefore expected to have a greater impact on individuals that have high leak respiration rates and low mitochondrial coupling, whose mitochondria may therefore need more oxygen to maintain ATP homeostasis (Salin et al., 2015; Salin et al., 2012). In this perspective, the link between whole-animal and mitochondrial performance may reflect the degree of mismatch between oxygen availability and oxygen requirements.

Studies of mammals show that feeding behaviour can be modulated by ATP homeostasis within the liver (Allen et al., 2009; Friedman, 1995; Rawson and Friedman, 1994). Lower hepatic ATP levels are associated with a stimulation in feeding behaviour (Rawson and Friedman, 1994; Rawson et al., 1996). Since feeding behaviour is regulated in fish in a very similar manner to that in mammals (Jordi et al., 2015; Lin et al., 2000), it is unclear why the brown trout with the lowest food intake in this study did not increase their intake when they clearly had the need to do so and had access to *ad libitum* food. One possible explanation for this is that locomotion and digestive activity require a substantial amount of energy since they involve ATP-dependent processes. Those fish that performed relatively badly had a high LEAK respiration and low RCR in their white muscle mitochondria, which *in vivo* would likely impair ATP production (Brand, 2005; Brand and Nicholls, 2011). Declines in ATP generation in skeletal muscle are known to affect locomotory capacity (Coen et al., 2012) and can explain the decrease in locomotor performance at increased temperatures in ectotherms (Bennett, 1990). While the consequences of individual differences in locomotor capacity for food intake would be significant for trout in their natural stream habitat (due to physical interactions during competition for access to food, necessity to move between feeding stations and to accelerate to intercept food items passing in rapid water currents, etc), they must have been virtually null in our experimental conditions where water flow rates were low and competition eliminated. Despite this, many individual fish in our warm, but otherwise benign laboratory conditions, were unable to eat enough to maintain their weight, suggesting that the effects we report here are more likely explained by poor digestive capacity. An decrease in mitochondrial coupling (i.e. lower RCR) within the intestine could have contributed to the low food intake, since this would have resulted in limited production of ATP for nutrient

absorption, nutrient transport and protein synthesis (Pelletier et al., 1994). Since the digestive track is involved in the central regulation of food intake (Jordi et al., 2015; Lin et al., 2000), the low food intake may also reflect a more general drop in intestinal function (Eya et al., 2012), but this requires further study.

The mitochondrial respiration rate of aquatic ectotherms is known to vary with temperature *in vitro* (Pörtner et al., 1999), and changes in water temperature can induce adjustments in mitochondrial respiratory capacities in fish (Chung and Schulte, 2015; Fangue et al., 2009; Iftikar and Hickey, 2013; Iftikar et al., 2014). Thus, an individual's ability to adjust its aerobic metabolism may be a more important component of its capacity to respond to environmental change than its metabolic performance in any one specific environment (Auer et al., 2015). However, data on individual variation in mitochondrial respiration are relatively scarce, so knowledge of the flexibility of these mitochondrial traits would be valuable for further investigating the effects of mitochondrial capacities on thermal tolerance.

A temperature of 19˚C is near the maximum that would naturally be encountered in the environment from which the study population of brown trout was obtained, and such temperatures do not generally persist for more than a few days (Maitland, 1963). Consequently, the current impact of natural thermal fluctuations on fitness may be relatively minor. However, our results suggest that in the context of global warming, some individuals perform much better than others if chronically exposed to an elevated thermal environment. Thus the potential consequences of such differences in performance for the abundance and distribution of ectotherms such as the brown trout may be profound (Hari et al., 2006). It has been suggested that mitochondrial respiratory capacities will determine the thermal range of ectothermic species (Blier et al., 2013). At an individual level, fish whose mitochondria are least able to perform at higher temperatures would likely be smaller due to stunted growth, have lower levels of energy storage, and ultimately have poorer survival and/or reproductive success (Carlson et al., 2008; Elliott and Elliott, 2010; Johnsson et al., 1999). This could also lead to differing habitat selection between conspecifics based on mitochondrial properties. As a consequence, we would expect a change not only in the distribution and abundance of populations in a warming environment, but also in the distribution of individuals based on their mitochondrial phenotype. Our results therefore provide insight into the potential mechanisms under selection in thermally challenging environments and population resilience to environmental change, as suggested by Schulte (2015).

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AUTHOR CONTRIBUTIONS

KS, SKA, CS, and NBM conceived the study. KS, SKA, GJA, performed the experiments, KS and SKA analysed the data. KS drafted the manuscript; SKA, CS and NBM revised the manuscript and GJA added some comments. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

DATA ACCESSIBILITY

Data reported in this publication will be stored in Dryad.

ETHICS STATEMENT

Experiments were approved by the University of Glasgow local ethical review panel, and all procedures were carried out under the jurisdiction of a UK Home Office project license (PPL 60/4292).

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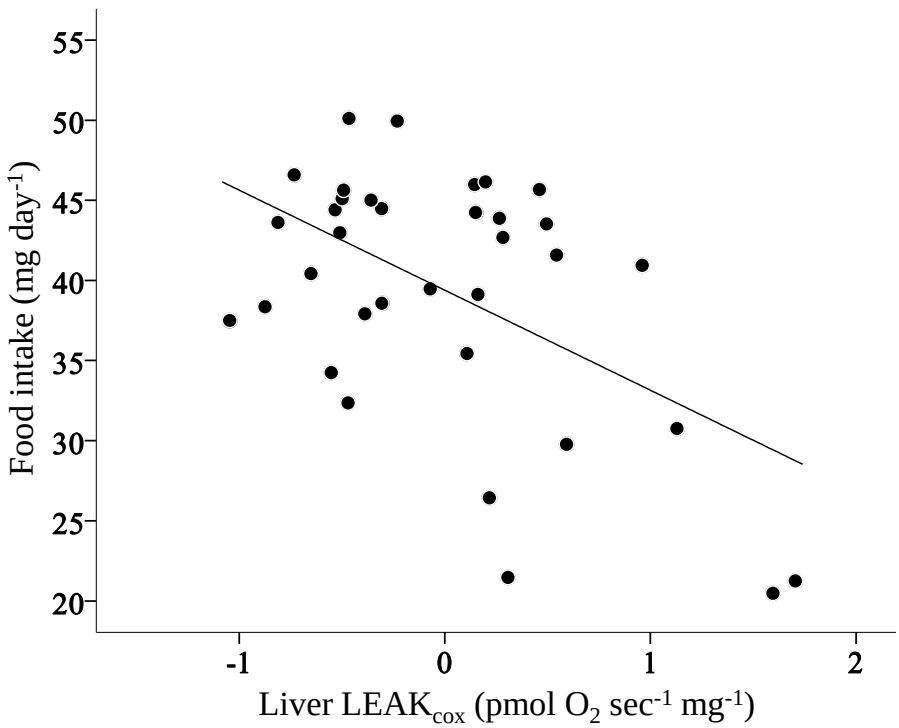
Figures

a.


55
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Food intake (mg day-1)
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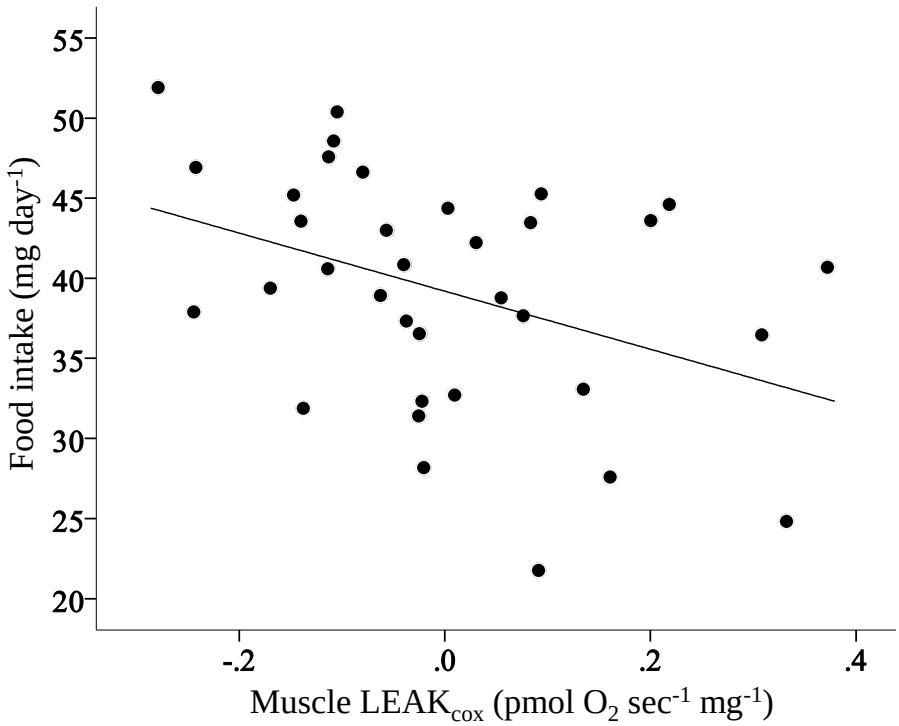
Liver LEAKcox (pmol O2 sec-1 mg-1)

b.


Food intake (mg day-1)
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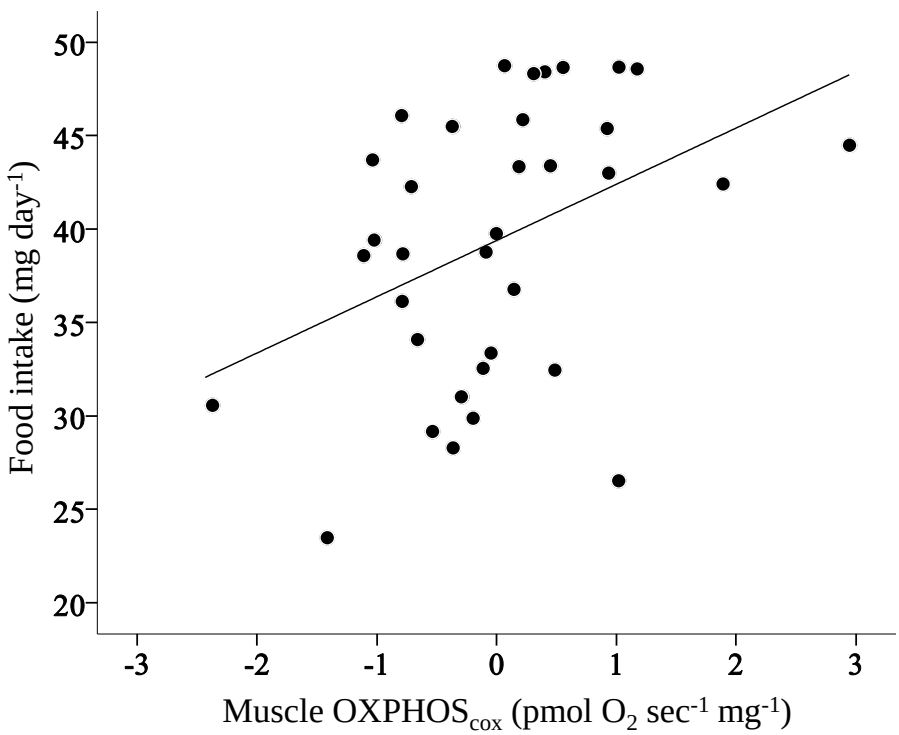
Muscle LEAKcox (pmol O2 sec-1 mg-1)

c.


Food intake (mg day-1)
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Muscle OXPHOScox (pmol O2 sec-1 mg-1)

d.

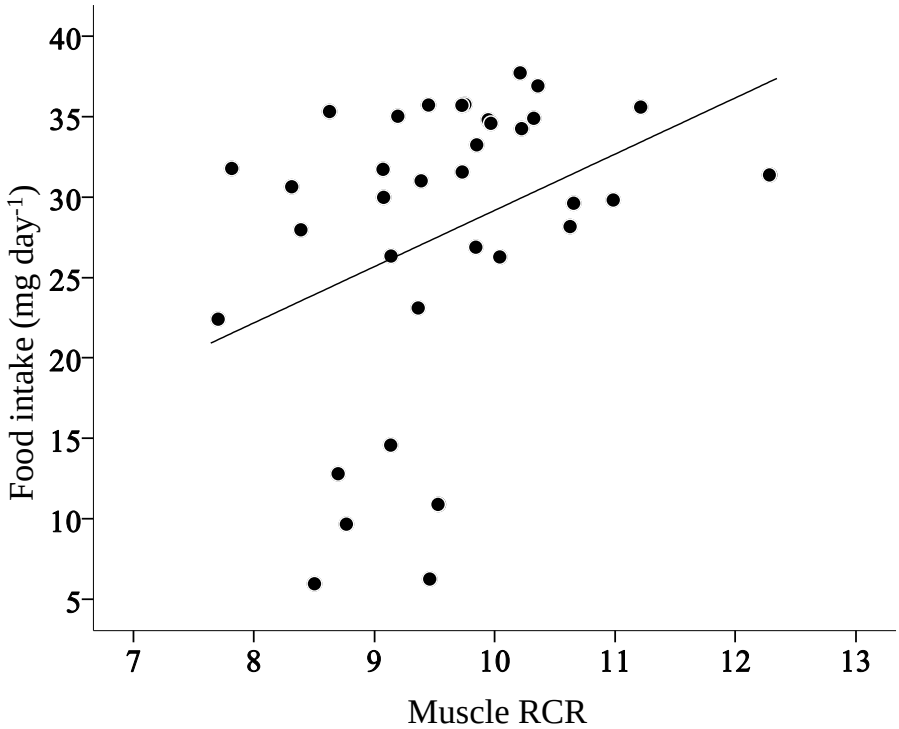

Figure 1: Relationships between food intake of brown trout (*Salmo trutta*, n = 35) maintained at 19˚C and a. their liver mitochondrial leak respiration, normalized for cytochrome oxidase (COX) activity, $LEAK_{cox}$ (η_p^2 = 0.204, p = 0.014), b. their muscle mitochondrial leak respiration, normalized for COX activity (η_p^2 = 0.135, p = 0.049), c. their muscle mitochondrial phosphorylative respiration normalized for COX activity, $OXPHOS_{cox}$ (η_p^2 = 0.143, p = 0.043), and d. their muscle mitochondrial respiratory control ration, RCR (η_p^2 = 0.119, p = 0.049). Values plotted on the y-axis are partial residuals evaluated at the mean body length of 85.57 mm and setting all other predictors equal to zero (see table 1 for details).

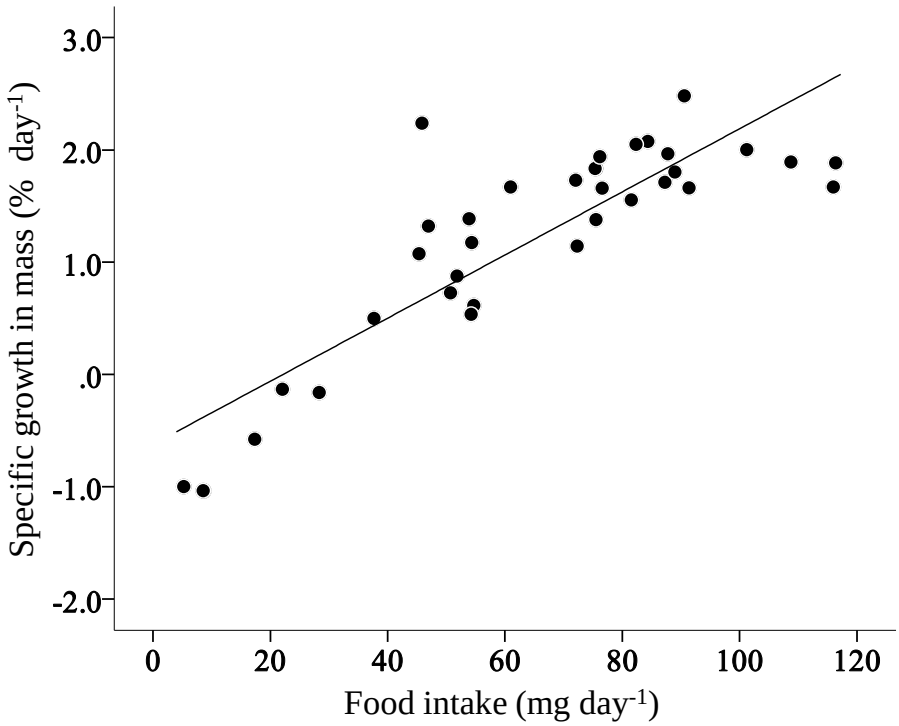

Figure 2: Relationship between specific growth of brown trout (*Salmo trutta*, n = 35) maintained at 19˚C and their food intake (Spearman $r^2 = 0.700$, $p < 0.001$).

Tables

Table 1: Results from linear regression analyses of food intake in brown trout (*Salmo trutta*, n = 35*)* as a function of body length and mitochondrial capacities (Model 1: Cytochrome oxidase (COX) activity, COX-normalized leak respiration rate ($LEAK_{cox}$), COX-normalized phosphorylating respiration rate ($OXPHOS_{cox}$); Model 2: Respiratory control ratio (RCR)). Bold denotes significance.

	Food intake			
Predictors	Parameter estimate ± 1SE	df	*t*	*p* value
Model 1				
Body length	1.991 ± 0.419	27	4.651	***<0.001***
Liver $LEAK_{cox}$	-12.517 ± 1.752	27	-2.634	***0.014***
Liver $OXPHOS_{cox}$	-0.842 ± 0.546	27	-0.171	0.135
Liver COX activity	-0.443 ± 0.371	27	-1.195	0.242
Muscle $LEAK_{cox}$	-35.985 ± 17.496	27	-2.057	***0.049***
Muscle $OXPHOS_{cox}$	6.113 ± 2.879	27	2.124	***0.043***
Muscle COX activity	-0.495 ± 0.563	27	-0.878	0.387
Model 2				
Body length	2.850 ± 0.406	31	7.702	***<0.001***
Liver RCR	0.888 ± 1.860	31	0.477	0.637
Muscle RCR	6.806 ± 3.323	31	2.048	***0.049***

Table 2: Descriptive statistics of the mass-specific mitochondrial respiration rates of liver and white muscle homogenates of brown trout *Salmo trutta* (n = 35). The substrates pyruvate, malate and succinate were present in all cases. Oxygen fluxes are expressed in pmol O_2 s^{-1} mg^{-1} wet mass and were measured at 19˚C.

	Tissue	**N**	**Min.**	**Max.**	**Mean**	**± SE**
Phosphorylating respiration without Cytochrome c	Muscle	35	10.85	22.04	14.68	0.37
	Liver	35	18.23	40.85	29.19	1.11
Phosphorylating respiration with Cytochrome c	Muscle	35	11.45	22.69	15.20	0.38
	Liver	35	20.25	42.41	31.09	1.10
Leak respiration	Muscle	35	1.18	2.63	1.60	0.05
	Liver	35	2.28	5.96	2.59	0.13
Respiratory Control Ratio	Muscle	35	7.70	12.28	9.58	0.16
	Liver	35	6.02	11.94	8.83	0.28
Cytochrome oxidase activity	Muscle	35	23.37	46.60	30.21	0.79
	Liver	35	36.20	89.39	55.46	2.05

Table 3: Results from linear regression analyses of specific growth rate (% change in mass per day) in brown trout (*Salmo trutta*, n = 35*)* as a function of mitochondrial respiratory capacities and food intake (Model 1: Cytochrome oxidase (COX) activity, COX-normalized leak respiration rate ($LEAK_{cox}$), COX-normalized phosphorylating respiration rate ($OXPHOS_{cox}$); Model 2: Respiratory control ratio (RCR)). Bold denotes significance.

Growth rate				
Predictors	Parameter estimate ± 1SE	df	*t*	*p* value
Model 1				
Food intake	0.026 ± 0.005	27	5.000	***<0.001***
Liver $LEAK_{cox}$	-0.206 ± 0.188	27	-1.096	0.283
Liver $OXPHOS_{cox}$	0.012 ± 0.020	27	0.605	0.550
Liver COX activity	-0.010 ± 0.013	27	-0.699	0.491
Muscle $LEAK_{cox}$	-0.478 ± 0.663	27	-0.720	0.477
Muscle $OXPHOS_{cox}$	0.016 ± 0.111	27	0.145	0.886
Muscle COX activity	-0.000 ± 0.020	27	-0.003	0.997
Model 2				
Food intake	0.028 ± 0.000	31	8.757	***<0.001***
Liver RCR	0.066 ± 0.052	31	1.273	0.212
Muscle RCR	0.031 ± 0.099	31	0.310	0.759